DOI:10.1111/j.1365-2125.2004.02225.x



# Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs

**Sven Björkman**

*Hospital Pharmacy, Malmö University Hospital, Malmö and Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden*

**Correspondence**
**Sven Björkman PhD,** Hospital Pharmacy, Malmö University Hospital, SE-205 02 Malmö, Sweden.
**Tel:** + 46 4033 3654
**Fax:** + 46 4033 6232
**E-mail:** sven.bjorkman@apoteket.se

**Keywords**
body composition, children, infants, midazolam, pharmacokinetics, theophylline, tissue distribution

**Received**
25 February 2004
**Accepted**
18 June 2004

**Aims**
To create a general physiologically based pharmacokinetic (PBPK) model for drug disposition in infants and children, covering the age range from birth to adulthood, and to evaluate it with theophylline and midazolam as model drugs.

**Methods**
Physiological data for neonates, 0.5-, 1-, 2-, 5-, 10- and 15-year-old children, and adults, of both sexes were compiled from the literature. The data comprised body weight and surface area, organ weights, vascular and interstitial spaces, extracellular body water, organ blood flows, cardiac output and glomerular filtration rate. Tissue: plasma partition coefficients were calculated from rat data and unbound fraction ($f_u$) of the drug in human plasma, and age-related changes in unbound intrinsic hepatic clearance were estimated from CYP1A2 and CYP2E1 (theophylline) and CYP3A4 (midazolam) activities *in vitro*. Volume of distribution ($V_{dss}$), total and renal clearance (CL and $CL_R$) and elimination half-life ($t_{1/2}$) were estimated by PBPK modelling, as functions of age, and compared with literature data.

**Results**
The predicted $V_{dss}$ of theophylline was 0.4-0.6 l kg$^{-1}$ and showed only a modest change with age. The median prediction error (MPE) compared with literature data was 3.4%. Predicted total CL demonstrated the time-course generally reported in the literature. It was 20 ml h$^{-1}$ kg$^{-1}$ in the neonate, rising to 73 ml h$^{-1}$ kg$^{-1}$ at 5 years and then decreasing to 48 ml h$^{-1}$ kg$^{-1}$ in the adult. Overall, the MPE was –4.0%. Predicted $t_{1/2}$ was 18 h in the neonate, dropping rapidly to 4.6-7.2 h from 6 months onwards, and the MPE was 24%. The predictions for midazolam were also in good agreement with literature data. $V_{dss}$ ranged between 1.0 and 1.7 l kg$^{-1}$ and showed only modest change with age. CL was 124 ml h$^{-1}$ kg$^{-1}$ in the neonate and peaked at 664 ml h$^{-1}$ kg$^{-1}$ at 5 years before decreasing to 425 ml h$^{-1}$ kg$^{-1}$ in the adult. Predicted $t_{1/2}$ was 6.9 h in the neonate and attained 'adult' values of 2.5-3.5 h from 1 year onwards.

**Conclusions**
A general PBPK model for the prediction of drug disposition over the age range neonate to young adult is presented. A reference source of physiological data was compiled and validated as far as possible. Since studies of pharmacokinetics in children present obvious practical and ethical difficulties, one aim of the work was to utilize maximally already available data. Prediction of the disposition of theophylline and midazolam, two model drugs with dissimilar physicochemical and pharmacokinetic characteristics, yielded results that generally tallied with literature data. Future use of the model may demonstrate further its strengths and weaknesses.

## Introduction

There is a paucity of data on the pharmacokinetics and pharmacodynamics of many drugs in infants and children [1, 2]. Consequently, dosing is often based on various arbitrary descriptors of body size. There is a growing understanding of the physiological and biochemical factors that affect drug disposition in children [2-5]. However, these findings must be translated into pharmacokinetic parameter values and then into dosing recommendations. This process requires the combining of patient-related and drug-related data from various sources, which can be done by physiologically based pharmacokinetic (PBPK) modelling [6]. As yet, the latter has been applied to children in only a few cases, first to predict body and organ exposure to environmental pollutants [7-9], and very recently [10] to compare the disposition of caffeine and theophylline in neonates and adults.

Comparative PBPK modelling of a drug in patients of various ages requires a realistic description of the physiology, in this case of infants and children, but no database or reference source containing all the necessary data has yet been published. Thus, children have been represented by generic models comprising four or five organ/tissue compartments: liver, (brain or kidney) and well-perfused, poorly perfused and adipose tissue [7–10]. A recent volume of the Annals of the International Commission on Radiological Protection (ICRP) [11] now gives comprehensive organ/tissue weight data for the neonate, 1-, 5-, 10- and 15-year-old children, and adults.

The aim of this study was to create a general PBPK model for drug disposition in infants and children, covering the age range from birth to adulthood, and to evaluate it with theophylline and midazolam as model drugs. Since studies of pharmacokinetics in children present obvious practical and ethical difficulties, a further aim of the work was to utilize maximally data already available in the literature.

## Methods

### *Physiological characteristics of organs and tissues*

*Body weight and surface area* Body weights (BW) were those compiled by the United States National Center for Health Statistics (1977) and cited in a recent textbook of paediatrics [12] and in part by Fomon *et al.* [13]. Other reference sources [3, 11, 14] give very similar values. Body surface area (BSA) was calculated from weights and heights given in the references [12, 13], using the formula of Haycock *et al.* [15].

*Organ/tissue weights* ICRP data were used for adults, and are assumed to be applicable to the age range 20–50 years [11]. In order to predict organ/tissue weights in children of ages other than those given in ICRP, the equations derived by Haddad *et al.* [3] were used, provided that these equations and the ICRP data gave similar estimates overall (<15% deviation). In some cases, a choice between these or other sources [13, 14, 16] had to be made based on biological plausibility and consistency of the total model (see Discussion).

*Blood flow and cardiac output* For most organs/tissues, specific blood flows (in l $min^{-1}$ per kg tissue) calculated from adult data [17] were adopted, as described previously [18]. Other sources were consulted for brain [19–21], muscle [22] and skin [22, 23]. Kidney blood flow as a function of age was estimated as described by Hayton [24], based on data from Rubin *et al.* [25]. Total liver blood flow ($Q_H$) in the infants and children was assumed to be proportional to BSA [26] and estimated from:

$$Q_{H(child)} = (BSA_{child}/BSA_{adult}) \times Q_{H(adult)} \qquad (1)$$

Blood flows to the liver and the prehepatic organs were calculated in relation to total liver blood flow, retaining the 'adult' [17] proportions between hepatic arterial flow and the respective blood flows through the stomach, intestine, spleen and pancreas. The sum of systemic blood flows is equal to the cardiac output (CO), values for which were obtained from the literature [11, 27, 28]. CO is often normalized to BSA and expressed as cardiac index (CI) in l $min^{-1}$ $m^{-2}$ [27].

*Vascular and interstitial spaces of organs/tissues* Data on the vascular spaces (*in vivo* blood content of the capillaries) and on interstitial spaces were compiled from earlier publications [18, 29–33]. The sum of capillary volumes should be a reasonably constant fraction of the total blood volume. The interstitial spaces, plus the plasma volume and the water contents of the gastrointestinal tract, should add up to the total extracellular water (ECW) volume. These estimates were compared with published values [11, 13, 14, 32, 34]. The fractional interstitial volumes of muscle and adipose tissue are considerably greater in infants and small children than in the adult [29, 31, 33]. Those of the other organs/tissues were assumed not to depend on age.

### *Drug distribution in blood*

Plasma protein binding of theophylline and midazolam in the infants and children were calculated from the age-related change in serum albumin concentration [35]. For theophylline, an unbound fraction ($f_u$) of 0.56 was assumed for adults [36]. A blood : plasma concentration

ratio (λ) of 0.82 has been determined [37]. This corresponds to an erythrocyte : plasma concentration ratio ($r_e$) of 0.56, consistent with the assumption that there is no binding of theophylline in the erythrocytes, i.e. that $r_e = f_u$. For midazolam, literature values of $f_u = 0.024$ [38] and λ= 0.80 [18] were used for adults.

*Tissue : plasma partition coefficients ($k_p$) and volume of distribution*

Tissue : plasma partition coefficients ($k_p$) were scaled from rat to humans according to a previously presented four-compartment model (Figure 1) [18] representing the anatomical structure of the tissues. In brief, the four compartments are cells, interstitial space, capillary plasma and capillary erythrocytes. The physiological parameters are the relative volumes of these compartments and the interstitial-to-plasma concentration ratio of albumin, whereas the drug-related parameters are the $r_e$ and the unbound fractions in plasma ($f_u$) and in the cells ($f_{u,cell}$). The parameter used for scaling of $k_p$ from rat data to humans is the $f_{u,cell}$, which is calculated from the measured $k_p$ in a model for the rat tissue and then applied in a corresponding model for the human tissue. The model equations are presented in reference [18].

Steady-state $k_p$ values for theophylline determined in rats [39] ranged between 0.55 and 0.68 in brain, heart, liver, kidneys, spleen and muscle, which in the four-compartment model for rat tissues gave calculated $f_{u,cell}$ values of 0.96–1.2. The unbound fraction of drug cannot exceed 1. Thus, there is no binding to intracellular constituents in these tissues. This was assumed for all tissues except lung and adipose tissue, for which the $k_p$ values were 0.61 and 0.07 [39], respectively. This corresponds to drug distribution to plasma, erythrocytes and the interstitial space only. In these tissues, the concentration in the intracellular compartment was assumed to be zero.

Scaling of the $k_p$ values of midazolam from rats to humans has been described previously [18]. New values were calculated in the present study since $f_u$ varied with age.

The volumes of distribution at steady state ($V_{dss}$) of the two drugs were calculated as:

$$V_{dss} = \sum_{i=1}^{n} k_{p,i} \times V_{tis,i} \qquad (2)$$

where $k_{p,i}$ and $V_{tis,i}$ are the $k_p$ and the physical volume of the $i^{th}$ out of $n$ organs/tissues. For blood, $k_p$ equals λ. For intestinal contents, the $k_p$ was assumed to equal $f_u$.

*Clearance*

Based on the urinary metabolite pattern after intravenous administration of theophylline [40, 41], clearance in adults was assumed to be 15% renal and 85% hepatic. The renal excretion of midazolam is negligible (<0.1%) [42]. Unbound intrinsic hepatic clearance ($CL_{u,i}$, ml min$^{-1}$ per kg liver) was calculated for both drugs in adult patients, using data from previous studies on theophylline [41, 43] and midazolam [18, 38, 44], according to the standard equation:

$$CL_{u,i} = (Q_H \times CL_{bl})/[f_{u,bl} \times (Q_H - CL_{bl})] \qquad (3)$$

where $Q_H$ is the estimated total hepatic blood flow, $CL_{bl}$ is hepatic clearance referenced to concentrations in whole blood, and $f_{u,bl}$ the unbound fraction in blood. $Q_H$ and liver weights were estimated for these subjects in the same way as in the final PBPK modelling. In all but one study $f_u$ was determined in plasma. The data from St-Pierre *et al.* [41] were used with the reference $f_u$ of 0.56 for theophylline. $f_u$ in plasma was converted to $f_{u,bl}$ by division with λ.

The hepatic metabolism of theophylline comprises 8-hydroxylation, to the metabolite 1,3-dimethyl uric acid

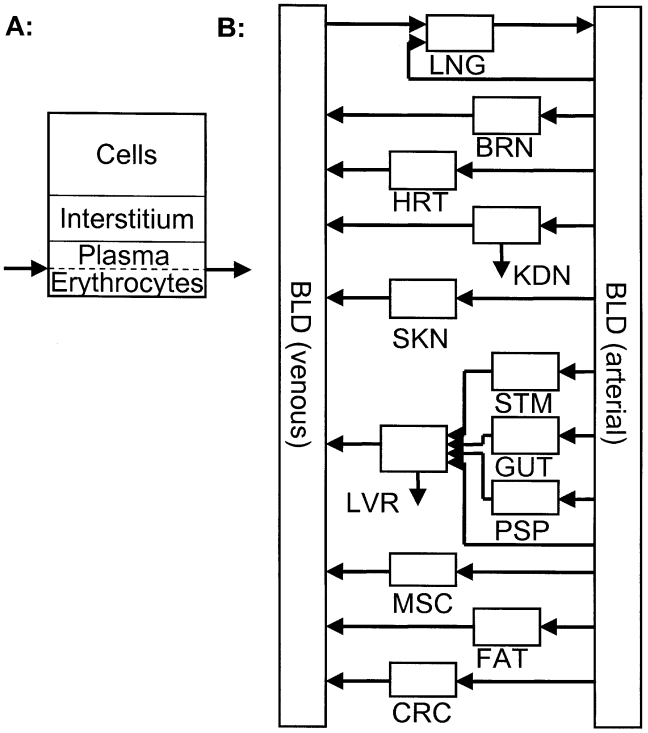


**Figure 1**

The compartmental structures of: (a) the single-tissue model used to scale $k_p$ values from rats to humans; (b) the perfusion-limited whole-body PBPK model. BLD, Blood; LNG, lungs; BRN, brain; HRT, heart; KDN, kidneys; SKN, skin; LVR, liver; STM, stomach; GUT, intestines; PSP, pooled spleen and pancreas; MSC, muscle; FAT, adipose tissue; CRC, 'carcass' (rest of the body)

(45% of total clearance) and demethylation in either the 1- or 3-position (40% of total clearance) [40, 41]. The former reaction is 80–90% catalysed by CYP1A2 and 10-20% by CYP2E1, whereas the latter pathways are catalysed almost exclusively by CYP1A2 [45, 46]. Thus, 92% of the total hepatic metabolism of theophylline in adults was assumed to be due to CYP1A2 and 8% to CYP2E1. *In vitro* activities (per mg of liver protein) of CYP1A2 and CYP2E1 have been quantified as a function of age in livers from infants and children [47, 48]. The demethylation of imipramine was used as a probe for CYP1A2 activity [48]. Fitting a biexponential growth function [5] to the original data (% of adult activity *vs.* mean age of the liver donors for each age group) gave the following age factors (age; % of adult activity): neonate, 5%; 6 months, 51%; 1 year, 63%; 2 years, 81%; and 5 years, 107%. Hydroxylation of chlorzoxazone was used as a probe for CYP2E1 activity [47]. Fitting the biexponential growth function to the data gave the estimates: neonate, 2%; 6 months, 43%; 1 year, 48%; 2 years, 57%; and 5 years, 75%. Midazolam metabolism as a function of age was estimated from *in vitro* data on CYP3A4 activity (testosterone 6β-hydroxylation) [49]. Fitting the biexponential growth function gave the estimated age factors: neonate, 10%; 6 months, 43%; 1 year, 54%; 2 years, 72%; and 5 years, 101%. From 10 years onward, normal adult enzyme activity was assumed for all three CYPs.

For each drug and age group, $CL_{u,i}$ per kg liver was calculated as the adult value multiplied by the age factor. Total hepatic CL was then calculated from $CL_{u,i}$, liver weight, $f_{u,bl}$ and $Q_H$ in each age (and sex) group. In the case of theophylline, this was done separately for the CYP2E1 and CYP1A2 isoforms and the values were added. One further elimination pathway of theophylline, methylation to caffeine, is of importance only in infants. Hepatic CL by this route in neonates has been estimated to be approximately 2.8 times greater than that due to metabolism by CYP1A2 plus CYP2E1 [10]. Thus, this extra CL was added. Based on urinary excretion of caffeine and its metabolites in proportion to theophylline metabolites [50], the increment in CL due to methylation was set to 10% in 6-month infants.

Finally, the hepatic extraction ratio ($E$) was calculated from:

$$E = CL_{bl}/Q_H \quad (4)$$

Renal clearance ($CL_R$) of unchanged theophylline was estimated from:

$$CL_{R(child)} = [(GFR_{child} \times fu_{(child)})/(GFR_{adult} \times fu_{(adult)})] \times CL_{R(adult)} \quad (5)$$

Data on glomerular filtration rate (GFR), measured as mannitol clearance, as a function of age were obtained from the literature [24]. $CL_{R(adult)}$ was 15% of total CL in the adult (see above). The fraction of theophylline excreted unchanged in the urine ($f_e$) was calculated by dividing $CL_R$ with total CL.

### *PBPK modelling*

The perfusion-limited recirculatory whole-body PBPK model (Figure 1) has been described previously [18]. The only novel feature is the incorporation of the renal clearance of theophylline. The model was developed using MATLAB software (version 6.0; MathWorks, Natick, MA, USA), with the SIMULINK graphical interface, on a Pentium III-based personal computer. The model comprised the arterial and venous blood, lungs, brain, heart, kidneys, skin, liver, stomach, intestines, pooled spleen and pancreas, muscle, adipose tissue, and 'carcass' compartments. Hard bone (bone mineral; BNE) was left out of the model, whereas the rest of the skeletal tissue, i.e. connective tissue and red bone marrow, was included in the 'carcass' compartment. The yellow, or fatty, bone marrow (BMA) was included in the adipose tissue compartment.

### *Pharmacokinetic data from children*

Data on the disposition of theophylline [50–57] and midazolam [58–61] in infants and children in various age groups were obtained from the literature. Studies were included only if the drug had been given intravenously, if plasma concentrations had been determined by a specific method, and if blood sampling and pharmacokinetic modelling were deemed adequate. Data from premature children were not included, nor were findings from studies in which other drugs, medical procedures or very severe illness could reasonably be expected to influence the disposition of the drug. Whenever possible, values of age, $V_{dss}$ (or in some cases $V_{d(area)}$), CL and terminal $t_{1/2}$ for individual patients were obtained from tables or read off graphs. However, in one study on theophylline, data were available only as mean ± SD [56], and in another, individual $t_{1/2}$ values were shown but there were only mean ± SD data for age [57]. In the studies on midazolam, data were generally only available as mean ± SD.

### *Statistical evaluation of the predictions*

The accuracy and precision of the predictions were tested by comparison with corresponding measured parameter values. In the case of theophylline the percent prediction error (PE) of $V_{dss}$, CL and $t_{1/2}$ was calculated from:

$$PE = 100 \times [(P - M)/M] \quad (6)$$

where $P$ is the predicted and $M$ is the measured value. The accuracy (bias) was estimated as the median prediction error (MPE) and the precision as the median absolute prediction error (MAPE) [62]. The 12 patients studied by Eriksson *et al.* [56], for whom no individual parameter data were given, were all given the mean parameter value.

The simulations on midazolam could not be evaluated in the same way, since individual parameter data were rarely given in the original publications. Instead, the simulated curves were compared with the mean ± SD of the published data.

## Results

The estimated body composition of the children and adults are shown in Table 1. The 'carcass' compartment comprised 8–16% of total body weight (11–16% in males and 8–12% in females). The estimated blood flow parameters are given in Table 2. The calculated was low in the neonates and was not related to age in the other groups. Table 3 shows the capillary and interstitial volumes of the organs/tissues. The blood volume in the capillaries amounted to 42–51% of total blood volume. Table 3 also lists the calculated $k_p$ values of the two drugs. For most organs/tissues there was a small progressive decrease in $k_p$ with age ($k_p$ values for ages between neonate and adult are not shown). This was due to a decrease in $f_u$ (Table 4). The $k_p$ values for muscle and adipose tissue were also influenced by the changes in interstitial *vs.* cell volume fraction. A lower interstitial volume and a higher percentage of fat in adipose tissue led to a lower $k_p$ for theophylline and a higher $k_p$ for midazolam. This is most apparent from the comparison of the values in the two highest age groups. In younger children the higher $f_u$ tends to obscure this effect.

The $CL_{u,i}$ of theophylline estimated from data in [41] and [43] was 50 and 53 ml $min^{-1}$ per kg liver, respectively. The value used in the PBPK modelling was 52 ml $min^{-1}$ $kg^{-1}$. The $CL_{u,i}$ values for midazolam, were nearly 400-fold higher: 19.3 [18], 21.7 [38] and 16.9 [44] l $min^{-1}$ $kg^{-1}$ liver. The intermediate value of 19.3 l $min^{-1}$ $kg^{-1}$ was used.

Table 4 lists the estimated pharmacokinetic parameters for theophylline and midazolam. The CL values were used as input data in the PBPK modelling, together with the organ/tissue weights, blood flows and $k_p$ values. The calculated hepatic extraction ratio ($E$) of both drugs

**Table 1**
Organ/tissue weights (kg), body weights and total extracellular water in both sexes (M/F) in the different age groups

| Organ | Neonate (M/F) | 6 months (M/F) | 1 year (M/F) | 2 years (M/F) | 5 years (M/F) | 10 years (M/F) | 15 years (M/F) | Adult (M/F) |
|---|---|---|---|---|---|---|---|---|
| BLD[a] | 0.16/0.15 | 0.35/0.31 | 0.48/0.42 | 0.54/0.49 | 0.74/0.72 | 1.44/1.25 | 2.23/1.72 | 3.07/2.08 |
| BRN | 0.35/0.35 | 0.75/0.71 | 0.94/0.87 | 1.12/1.03 | 1.29/1.19 | 1.36/1.25 | 1.39/1.28 | 1.45/1.30 |
| HRT | 0.02/0.02 | 0.04/0.04 | 0.05/0.05 | 0.07/0.06 | 0.09/0.09 | 0.15/0.15 | 0.26/0.23 | 0.33/0.25 |
| LNG | 0.06/0.05 | 0.12/0.12 | 0.16/0.17 | 0.24/0.24 | 0.34/0.32 | 0.43/0.50 | 0.90/0.75 | 1.20/0.95 |
| LVR | 0.12/0.13 | 0.27/0.25 | 0.36/0.34 | 0.48/0.46 | 0.59/0.59 | 0.87/0.89 | 1.35/1.33 | 1.80/1.40 |
| KDN | 0.03/0.03 | 0.05/0.04 | 0.06/0.06 | 0.09/0.08 | 0.11/0.10 | 0.18/0.18 | 0.25/0.24 | 0.31/0.28 |
| SKN | 0.17/0.16 | 0.29/0.27 | 0.34/0.32 | 0.41/0.39 | 0.55/0.53 | 0.80/0.81 | 2.01/1.66 | 3.30/2.30 |
| STM | 0.01/0.01 | 0.02/0.02 | 0.02/0.02 | 0.03/0.03 | 0.05/0.05 | 0.09/0.09 | 0.14/0.14 | 0.15/0.14 |
| GUT | 0.05/0.05 | 0.09/0.09 | 0.14/0.14 | 0.19/0.19 | 0.34/0.34 | 0.58/0.58 | 0.82/0.82 | 1.02/0.96 |
| - cnt[b] | 0.14/0.14 | 0.23/0.23 | 0.24/0.24 | 0.30/0.30 | 0.30/0.30 | 0.42/0.42 | 0.74/0.74 | 0.90/0.85 |
| PSP | 0.02/0.01 | 0.03/0.03 | 0.05/0.05 | 0.07/0.07 | 0.09/0.09 | 0.14/0.15 | 0.24/0.23 | 0.29/0.25 |
| BNE | 0.17/0.17 | 0.40/0.38 | 0.59/0.59 | 0.85/0.82 | 1.26/1.26 | 2.30/2.30 | 4.05/3.70 | 5.50/4.00 |
| BMA | 0.00/0.00 | 0.00/0.00 | 0.02/0.02 | 0.03/0.03 | 0.16/0.16 | 0.63/0.63 | 1.48/1.38 | 2.48/1.80 |
| MSC | 0.80/0.80 | 1.35/1.35 | 1.90/1.90 | 2.83/2.83 | 5.60/5.60 | 11.0/11.0 | 24.0/17.0 | 29.0/17.5 |
| FAT | 0.89/0.89 | 2.97/2.85 | 3.64/3.23 | 3.76/3.72 | 5.00/5.00 | 7.50/9.00 | 9.50/16.0 | 14.5/19.0 |
| CRC | 0.56/0.37 | 1.08/0.56 | 1.20/0.76 | 1.62/1.18 | 2.18/1.35 | 3.51/3.41 | 7.35/6.48 | 7.70/6.95 |
| BW[c] | 3.55/3.33 | 8.03/7.25 | 10.2/9.18 | 12.6/11.9 | 18.7/17.7 | 31.4/32.6 | 56.7/53.7 | 73.0/60.0 |
| ECW[d] | 38/39 | 27/27 | 29/29 | 29/29 | 27/26 | 23/23 | 22/22 | 21/20 |

[a]*Excluding blood in capillary spaces in the organs.* [b]*Gut contents.* [c]*Body weight (kg).* [d]*Extracellular water (% of BW).*

**Table 2**
Organ blood flows (l $min^{-1}$ $kg^{-1}$ tissue), cardiac output, cardiac index, and total hepatic blood flow in the different age groups

| Organ | Neonate (M/F) | 6 months (M/F) | 1 year (M/F) | 2 years (M/F) | 5 years (M/F) | 10 years (M/F) | 15 years (M/F) | Adult (M/F) |
|---|---|---|---|---|---|---|---|---|
| BRN | 0.50/0.50 | 0.70/0.70 | 0.70/0.70 | 0.70/0.70 | 0.81/0.81 | 0.68/0.68 | 0.54/0.52 | 0.54/0.52 |
| HRT | | | | 0.79/1.08 | (in all age groups) | | | |
| LNG[a] | | | | 0.16/0.16 | (in all age groups) | | | |
| LVR[b] | 0.59/0.57 | 0.49/0.47 | 0.41/0.39 | 0.36/0.35 | 0.32/0.30 | 0.28/0.28 | 0.28/0.27 | 0.25/0.25 |
| KDN | 1.34/1.08 | 2.82/2.36 | 2.97/2.53 | 2.93/2.52 | 3.43/2.79 | 3.94/2.78 | 4.21/3.25 | 4.00/3.49 |
| SKN | 0.16/0.16 | 0.16/0.16 | 0.16/0.16 | 0.16/0.16 | 0.16/0.16 | 0.16/0.16 | 0.12/0.12 | 0.12/0.12 |
| STM | 1.25/1.13 | 1.05/0.94 | 0.88/0.78 | 0.77/0.70 | 0.68/0.60 | 0.60/0.56 | 0.60/0.54 | 0.53/0.50 |
| GUT | 2.11/2.05 | 1.78/1.70 | 1.49/1.41 | 1.31/1.27 | 1.15/1.09 | 1.02/1.02 | 1.01/0.97 | 0.90/0.91 |
| PSP | 2.18/2.11 | 1.85/1.75 | 1.56/1.45 | 1.36/1.29 | 1.18/1.11 | 1.06/1.05 | 1.07/1.00 | 0.93/0.92 |
| BNE | | | | 0/0 | (in all age groups) | | | |
| BMA | | | | 0.024/0.026 | (in all age groups) | | | |
| MSC | | | | 0.039/0.039 | (in all age groups) | | | |
| FAT | | | | 0.024/0.026 | (in all age groups) | | | |
| CRC | | | | 0.084/0.090 | (in all age groups) | | | |
| CO[c] | 0.58/0.55 | 1.35/1.22 | 1.68/1.52 | 2.06/1.90 | 2.88/2.63 | 3.90/3.74 | 5.77/5.15 | 6.79/5.69 |
| CI[d] | 2.55/2.52 | 3.41/3.31 | 3.57/3.46 | 3.69/3.54 | 3.81/3.62 | 3.58/3.36 | 3.55/3.32 | 3.59/3.44 |
| $Q_H$ [e] | 0.22/0.21 | 0.38/0.35 | 0.45/0.41 | 0.53/0.50 | 0.72/0.68 | 1.04/1.04 | 1.55/1.45 | 1.72/1.52 |

*[a]Bronchial artery. [b]Hepatic artery. [c]Cardiac output (l $min^{-1}$). [d]Cardiac index (l $min^{-1}$ $m^{-2}$). [e]Total hepatic blood flow (l $min^{-1}$).*

increased with age during the period of liver enzyme maturation. That of theophylline remained very low in all age groups, whereas that of midazolam was low in the infants and rose into the intermediate range in children and adults.

Predicted plasma concentration curves of theophylline are shown in Figure 2, which also demonstrates the close agreement between model-predicted and measured plasma concentrations in male adults. Figure 3 shows $V_{dss}$, CL and the terminal $t_{1/2}$ predicted by the PBPK modelling as functions of age, in comparison with literature data. The MPE for the prediction of $V_{dss}$ was 3.4% (range −25–83) and the MAPE 5.2% (1.8–83) ($n = 30$). For the prediction of CL, the MPE was −4.0% (−56–212) and the MAPE 23% (1.2–212) ($n = 104$). Finally, the MPE for the prediction of $t_{1/2}$ was 24% (−35–575) and the MAPE 24% (1.9–575) ($n = 43$).

Predicted plasma concentration curves for midazolam are shown in Figure 4, which also demonstrates the good agreement between simulated curves for the 2–10-year-olds and mean concentration data from children who had received midazolam as premedication before minor hospital procedures [61]. Finally, Figure 5 shows $V_{dss}$, CL and the terminal $t_{1/2}$ as functions of age, in comparison with literature data.

For both drugs, predicted differences between the sexes were modest. The calculated $V_{dss}$ followed the amount and characteristics of adipose tissue, being higher in neonates than in older infants for the hydrophilic drug theophylline, and lower for the lipophilic drug midazolam. CL per kg BW was maximal and $t_{1/2}$ minimal at 2–5 years. The changes in $t_{1/2}$ with age were chiefly due to changes in CL.

## Discussion

Creating a comprehensive table of organ/tissue weights in human beings requires compilation of data of varying quality from many sources. Obtaining weights of brain, heart, lungs, liver, kidneys, stomach, spleen and pancreas presented no particular problems, since these have been determined by direct dissection and weighing [3, 11, 32]. However, some organ/tissue volumes must be determined indirectly. Total blood volume as a function of age has been measured using $^{125}$iodinated serum albumin [16]. The weight of skin has been estimated as BSA multiplied by epidermal plus dermal skin mass thickness in mg $cm^{-2}$ [11]. According to ICRP, skin thickness remains constant over the ages of 0–10 years and then increases markedly during the next 10 years. This is reflected as a sharp rise in skin mass between the ages of 10 and 15 (Table 1). The weight of hard bone is

**Table 3**
Capillary and interstitial spaces in the organs/tissues and calculated tissue:plasma partition coefficients ($k_p$) for theophylline and midazolam

| Organ | Capillary space (fraction) | Interstitial space (fraction) | Theophylline $k_p$ | Midazolam $k_p$ |
|---|---|---|---|---|
| BRN | 0.022 | 0.10 | 0.65; 0.59[a] | 1.3; 1.1[a] |
| HRT | 0.16 | 0.12 | 0.68; 0.63[a] | 2.0; 1.6[a] |
| LNG | 0.53 | 0.20 | 0.61; 0.59[a] | 2.0; 1.7[a] |
| LVR | 0.14 | 0.17 | 0.69; 0.63[a] | 1.9; 1.6[a] |
| KDN | 0.23 | 0.20 | 0.71; 0.66[a] | 1.7; 1.4[a] |
| SKN | 0.025 | 0.30 | 0.74; 0.70[a] | 0.72; 0.63[a] |
| STM | 0.040 | 0.10 | 0.67; 0.61[a] | 3.0; 2.4[a] |
| GUT | 0.040 | 0.10 | 0.67; 0.61[a] | 1.9; 1.5[a] |
| PSP | 0.33 | 0.10 | 0.72; 0.67[a] | 1.2; 1.0[a] |
| BNE | 0 | 0 | 0; 0[a] | 0; 0[a] |
| BMA | As for FAT | As for FAT | As for FAT | As for FAT |
| MSC | | | | |
| Neonate | 0.026 | 0.33 | 0.70 | 0.56 |
| 6 months | 0.026 | 0.27 | 0.69 | 0.56 |
| 1 year | 0.026 | 0.26 | 0.68 | 0.55 |
| 2 years | 0.026 | 0.25 | 0.68 | 0.54 |
| 5 years | 0.026 | 0.21 | 0.65 | 0.51 |
| 10 years | 0.026 | 0.16 | 0.61 | 0.46 |
| 15 years | 0.026 | 0.16 | 0.61 | 0.45 |
| Adult | 0.026 | 0.16 | 0.61 | 0.45 |
| FAT | | | | |
| Neonate | 0.018 | 0.54 | 0.46 | 2.0 |
| 6 months | 0.018 | 0.21 | 0.19 | 3.1 |
| 1 year | 0.018 | 0.28 | 0.24 | 2.9 |
| 2 years | 0.018 | 0.30 | 0.26 | 2.7 |
| 5 years | 0.018 | 0.28 | 0.24 | 2.6 |
| 10 years | 0.018 | 0.21 | 0.18 | 2.6 |
| 15 years | 0.018 | 0.16 | 0.14 | 2.6 |
| Adult | 0.018 | 0.10 | 0.093 | 2.8 |
| CRC | 0.026 | 0.35 | 0.76; 0.72[a] | 0.74; 0.66[a] |

[a]*In neonates and adults.*

calculated from bone mineral weights obtained in measurements of chemical body composition [11]. The ICRP data were adopted, and bone weights at 0.5 and 2 years were calculated from the bone mineral weights given by Butte *et al.* [14], assuming the same ratio of bone to mineral weight as in the 1-year-old.

Estimates of muscle mass are based on urinary excretion of creatinine (which is assumed to be proportional to total muscle mass at all ages). ICRP data from 1975 [32], cited by Haddad *et al.* [3], and ICRP data from 2003 [11] are very different, giving values of 2 kg for the 5-year-old in 1975 and 5.6 kg in 2003. The latter data were used, with linear interpolations between 0 and 1 and 1 and 5 years. Adipose tissue weights have been estimated from measurements of body composition, i.e. as total weight of body fat divided by percentage fat in adipose tissue [11]. ICRP [11] data for anatomically distinct ('separable') adipose tissue were adopted for adults and ages 0, 5, 10 (boys only) and 15 years. For the age group 0.5–2 years, total weight of adipose tissue was calculated from other published data on body fat [13, 14] and percentage fat in adipose tissue [11, 31–33]. 'Separable adipose tissue' was assumed to represent 95% of total adipose tissue, in accordance with the ICRP data for 0 and 1 year. For 10-year-old children, ICRP postulates the same weight of adipose tissue in boys and girls. However, since girls seem to have a higher percentage of body fat already at this age [13],

**Table 4**
Calculated pharmacokinetic parameters of theophylline and midazolam in the different age groups: unbound fraction in plasma ($f_u$), volume of distribution at steady state ($V_{dss}$), hepatic and renal clearances ($CL_H$ and $CL_R$), hepatic extraction ratio ($E$), fraction excreted ($f_e$) and terminal half-life ($t_{1/2}$)

| Parameter | Neonate (M/F) | 6 months (M/F) | 1 year (M/F) | 2 years (M/F) | 5 years (M/F) | 10 years (M/F) | 15 years (M/F) | Adult (M/F) |
|---|---|---|---|---|---|---|---|---|
| *Theophylline* | | | | | | | | |
| $f_u$ (%) | 62 | 62 | 62 | 61 | 59 | 57 | 56 | 56 |
| $V_{dss}$ (l) | 2.2/2.0 | 3.8/3.3 | 5.0/4.5 | 6.5/6.0 | 9.5/8.8 | 15/15 | 28/23 | 34/25 |
| $CL_H$ (ml min$^{-1}$) | 0.73/0.73 | 5.5/5.1 | 7.0/6.6 | 12/11 | 18/18 | 25/26 | 38/38 | 51/40 |
| $E$ (%) | 0.42/0.45 | 1.8/1.9 | 2.0/2.0 | 2.8/2.8 | 3.2/3.4 | 3.0/3.1 | 3.1/3.3 | 3.7/3.3 |
| $CL_R$ (ml min$^{-1}$) | 0.44/0.41 | 1.4/1.2 | 2.0/1.8 | 2.7/2.6 | 3.7/3.5 | 5.0/5.2 | 7.5/7.1 | 8.8/7.2 |
| $f_e$ (%) | 38/36 | 20/19 | 22/21 | 19/18 | 17/16 | 17/17 | 16/16 | 15/15 |
| $t_{1/2}$ (h) | 19/18 | 6.0/5.5 | 6.1/6.1 | 5.1/5.0 | 5.0/4.6 | 5.7/5.7 | 7.2/6.1 | 6.7/6.1 |
| *Midazolam*[a] | | | | | | | | |
| $f_u$ (%) | 3.1 | 3.1 | 3.0 | 2.9 | 2.7 | 2.5 | 2.4 | 2.4 |
| $V_{dss}$ (l) | 4.0/3.8 | 14/13 | 16/14 | 17/17 | 23/22 | 35/39 | 55/67 | 79/81 |
| $CL_H$ (ml min$^{-1}$) | 7.1/7.1 | 56/52 | 86/81 | 134/128 | 203/200 | 276/280 | 416/402 | 519/423 |
| $E$ (%) | 4.1/4.4 | 19/19 | 24/25 | 32/32 | 35/37 | 33/34 | 34/35 | 38/35 |
| $t_{1/2}$ (h) | 7.1/6.8 | 4.3/4.2 | 3.5/3.3 | 2.8/2.7 | 2.6/2.5 | 2.6/2.7 | 2.7/3.1 | 3.0/3.5 |

[a]*For midazolam, $CL_R$ and $f_e$ are negligible.*

another 1.5 kg of adipose tissue was added, bringing the 'carcass' weight to a similar value in the two sexes. Yellow (fatty) bone marrow is not developed to any appreciable extent at ages below 1 year. Weights according to ICRP [11] were used.

There is a lack of published data on capillary and interstitial spaces in humans. Even the ICRP [32] mainly cites findings from animal studies. Total extracellular space has been measured as chloride ion distribution space in human postmortem samples of muscle, skin, heart, liver, kidney and brain [29, 30]. The average extracellular space in muscle was 35% in neonates, 29% in 4–7-month-old infants and 18% in adults (including one 11- and one 16-year-old boy) [29]. In Table 3, the extracellular space has been divided into capillary plasma space (60% of total capillary space; assumed unchanged with age) and interstitial space, with interpolation between 6 months and 10 years. No clear changes of extracellular space with age were found in the other investigated organs [30]. For adipose tissue, the changes in interstitial space with age were calculated from data on lipid content and fat cell number [31–33]. Estimates of capillary and interstitial space in the 'carcass' compartment are clearly arbitrary. Vascular space is assumed to be similar to that in muscle and skin, whereas a high extracellular water content is postulated, since 'carcass' encompasses several body fluid compartments such as the lymphatic system, red bone marrow, glands, joints, and pleural and abdominal cavities. Plasma, interstitial water and gut contents should add up to the total extracellular water (ECW). The estimated values (Table 1) lie within the range of data reported in the literature [13, 14, 32, 34] and illustrate the high ECW content of the neonate followed by an initially steep decline towards the adult values of 20–22%.

Changes in regional blood flows with age are well documented for kidneys [25] and have been investigated to some extent for brain [19–21]. Very limited data were found for muscle and skin [22, 23]. Otherwise, reported 'adult' data were used for all organs except those of the splanchnic circulation. The assumption that $Q_H$ changes in proportion to BSA is based on the finding that indocyanine green clearance in ml min$^{-1}$ per m$^2$ BSA showed no correlation with age over the period 0.9–30 years [26]. In addition, the Haycock BSA formula [15] corresponds to an allometric exponent of 0.71 (i.e. BSA is proportional to $BW^{0.71}$), and thus the estimated $Q_H$ would also have this relationship to BW. The allometric exponent for $Q_H$ in scaling across animal species is reported to be 0.89 [63]. Therefore, it is reasonable to assume that $Q_H$ is approximately proportional to BW to

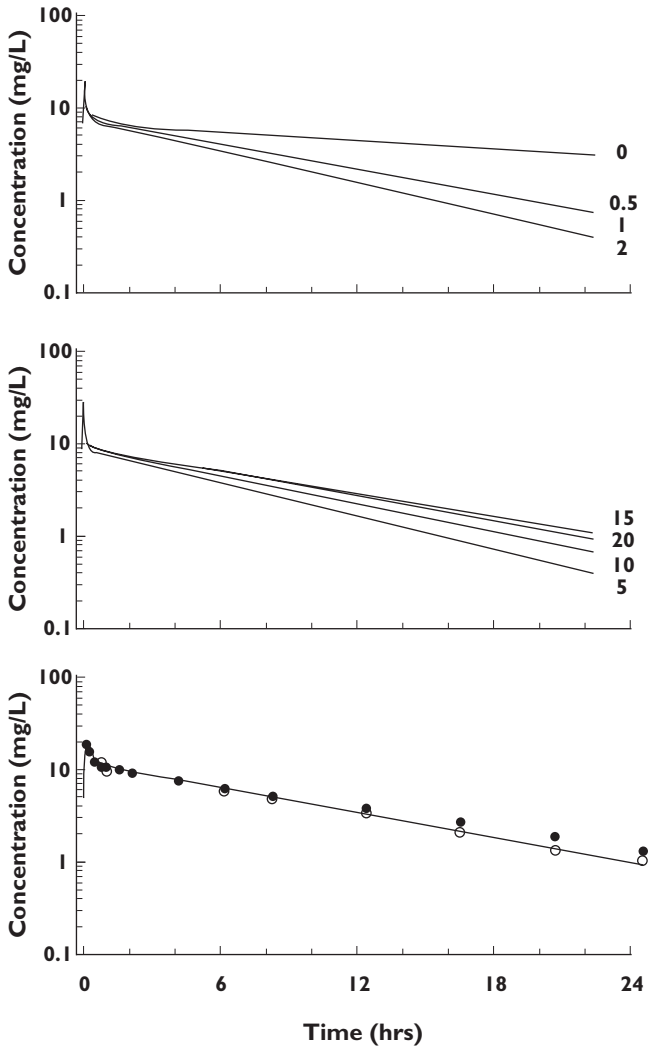


**Figure 2**

Predicted arterial concentration-time curves of theophylline in male patients after intravenous infusion of 4 mg kg$^{-1}$ over 5 min. Predicted concentration curves for females were similar (see $t_{1/2}$ values in Table 4). The numbers to the right of the plots denote ages (years) of the hypothetical subjects. The lowest panel shows the predicted arterial plasma concentration-time curve in a male adult after intravenous infusion of 6 mg kg$^{-1}$ of theophylline over 15 min, together with concentration data from St-Pierre *et al.* [41]. ●, Dose given at 20.00 h; ○, dose given at 08.00 h. Mean values, $n = 8$ in both cases

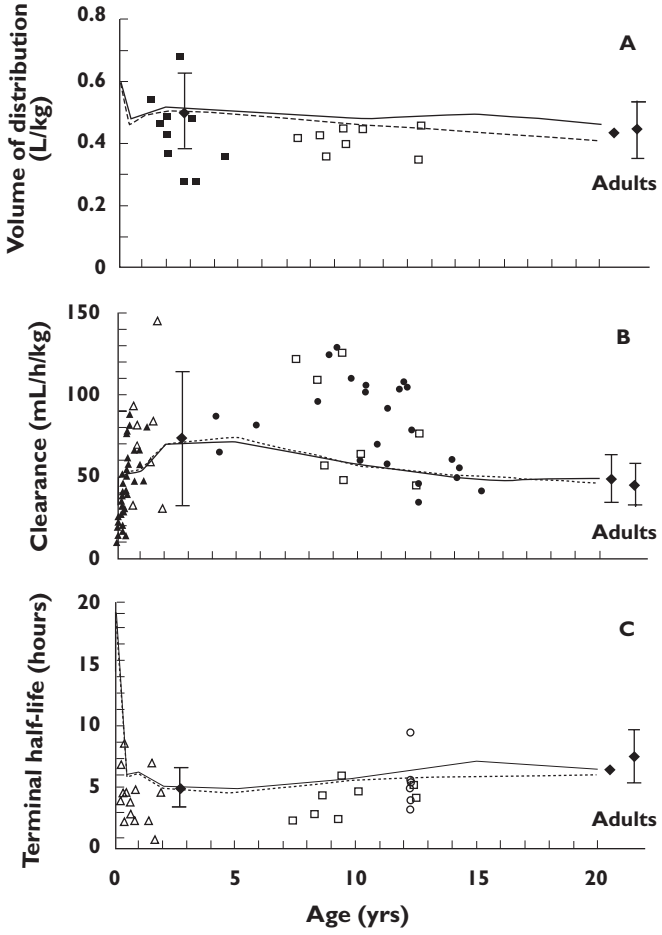


**Figure 3**

Predicted pharmacokinetic parameters for theophylline as functions of age, together with literature data. ----, Males; - - - -, females. (a) Volume of distribution at steady state ($V_{dss}$). (b) Clearance (CL). (c) Terminal half-life ($t_{1/2}$). Data sources: (▲) [50]; (■) [51]; (●) [52]; (Δ) [53]; (□) [55]; (○) [57], mean ± SD, (actual age range 9 months to 5 years) [56], adults [41] and [43]

the power of 0.7–0.9. Our current state of knowledge is far from satisfactory and the practical and ethical difficulties in obtaining accurate, comprehensive blood flow data are obvious. In the present case errors in the estimate of $Q_H$ have little influence on calculations of CL for a low-extraction drug like theophylline, but may become more critical for a medium-extraction drug like midazolam. However, in infants, due to the low activity of CYP3A4, midazolam also becomes a low-extraction drug (see Table 4).

Finally, the sum of the regional blood flows should equal CO. In the adult, resting CO is 5.5–7 l min$^{-1}$, corresponding to a CI of about 3.5–4 l min$^{-1}$ m$^{-2}$ [11, 27]. The calculated values in Table 2 for infants 0–1 year old correspond fairly well to a measured average CI in infants of 2.6 l min$^{-1}$ m$^{-2}$ at 0.2–2 months rising to 3.2 l min$^{-1}$ m$^{-2}$ at 11–14 months [28]. Estimates for infants and children vary widely between other sources [11, 12, 27]. However, CO is closely related to metabolic rate, and it is reasonable to assume that resting CO (like $Q_H$) relates allometrically to BW [27]. If this is the case, and the allometric exponent is close to that of

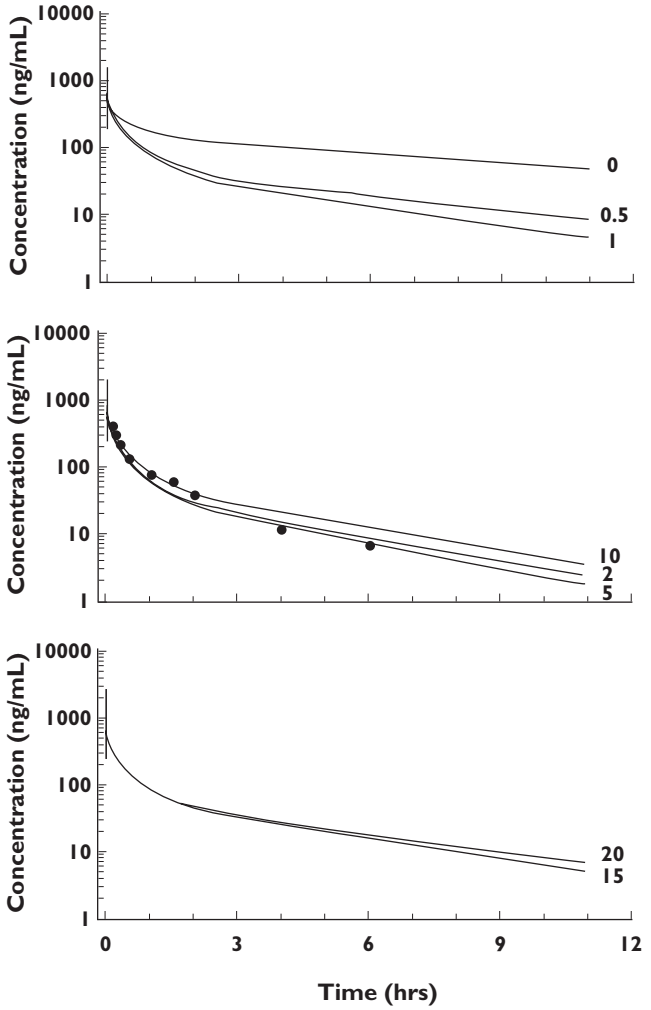


**Figure 4**
Predicted arterial concentration-time curves for midazolam in female patients after intravenous infusion of 0.2 mg kg$^{-1}$ over 1 min. Predicted curves for males were similar (see $t_{1/2}$ values in Table 4). The numbers to the right of the plots denote ages (years) of the hypothetical subjects. Data points in the middle panel are mean plasma concentrations in 14 patients with a mean age of 6.2 years [61], adjusted for dose (from 0.15 to 0.20 mg kg$^{-1}$). The model has previously been validated for middle-aged and elderly patients [18]

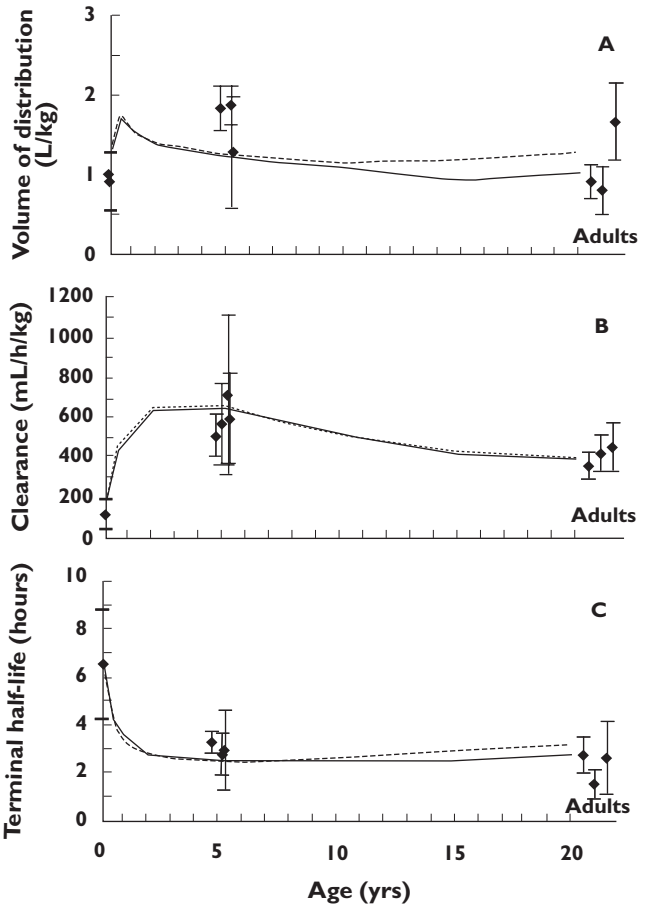


**Figure 5**
Predicted pharmacokinetic parameters for midazolam as functions of age, together with literature data. ——, Males; - - - -, females. (a) Volume of distribution at steady state ($V_{dss}$). (b) Clearance (CL). (c) Terminal half-life ($t_{1/2}$). Data sources: neonates [59, 60], 4.7 (± 2.6) years, 4.9 (± 2.3) years, 5.2 (± 2.5) years [58], 5.2 (± 2) years [61], adults [18] [38], and [44]. Horizontal error bars (for age) are omitted for clarity

BSA, then CI should be fairly constant over age. The estimates for the age range 6 months to adult in Table 2 are in agreement with this assumption.

The technique of PBPK modelling was different for distribution and elimination. The former was predicted from first principles, i.e. from animal $k_p$ data, $f_u$ and organ/tissue composition in all age groups. The previously published [18] four-compartment model for the calculation of $k_p$ allows adjustment of this parameter for changes in interstitial (or any fractional) volume in a tissue. This became a very useful feature in the present work, since $k_p$ is an age-dependent parameter, related to both tissue composition and $f_u$.

In contrast to $V_{dss}$, hepatic and renal CL were predicted by 'retrograde' modelling from adult data. Instead of calculating CL values in infants and children from *in vitro* metabolism data or based on the mechanism of renal excretion, findings from studies in adults were taken as points of departure. The PBPK modelling was then used to estimate changes with decreasing age. The assumptions on which this retrograde modelling are based, e.g. that $CL_{u,i}$ in ml min$^{-1}$ per kg liver can be calculated from published values of total CL, are the same as in the 'forward' modelling, i.e. the prediction of total CL from $CL_{u,i}$ assuming a certain $Q_H$, liver

weight and $f_u$. The publications [18, 38, 41, 43, 44] chosen for the calculation of $CL_{u,i}$ presented the necessary data and are representative of studies in human subjects.

The choice of retrograde modelling of CL was due to the paucity of quantitative data on the hepatic metabolism of theophylline or midazolam, or indeed of any drug, as a function of age, and to the inherent difficulties in estimating *in vivo* CL from *in vitro* experiments. The principle has been described by Alcorn and McNamara [4, 5]. However, these authors defined and used an 'infant scaling factor' for *in vivo* CL, whereas in the present study growth functions were fitted to the *in vitro* enzyme activity data. The remainder of the scaling, i.e. for liver weight, $Q_H$ and $f_u$, was part of the PBPK model. In both cases, the modelling assumes a linear CL (i.e. first-order elimination) in all age groups. The enzyme data used here [47–49] are the only activities published for CYP1A2, CYP2E1 and CYP3A4 as functions of age in infants. They cover the first year of life in reasonable detail, whereas activities at ages above 1 year are not well described. That full enzyme activity has not been attained at 2 years is supported by the finding [64] that an 'adult' pattern of theophylline metabolite excretion is not observed until 3 years of age or more.

Ginsberg *et al.* [10] described a basic PBPK model (comprising liver, kidney, well-perfused and poorly perfused organs and fat) for theophylline and caffeine in neonates and adults. Hepatic clearances of the two drugs in the neonate were calculated by the 'infant scaling factor' approach [4, 5] as well as by fitting the model to *in vivo* CL data, and the estimates were compared. Although the model gave a good description of the disposition of theophylline in the neonate, it failed to predict accurately $t_{1/2}$ in adults.

Whereas pharmacokinetic data on theophylline were plentiful, they were more scarce for midazolam. In some cases the predictions had to be compared with literature data that did not strictly meet the inclusion criteria listed in Methods. Data in neonates were available only from critically ill patients [59, 60]. Parameter values for midazolam were generally also presented only for a group of patients. These values were judged as acceptable for model verification if the predicted curves were comparatively flat over the pertinent age range.

The calculated $V_{dss}$ of theophylline was in reasonable agreement with data from the literature, with a median bias of only 3.4% (i.e. a slight tendency towards overprediction) and a median imprecision of 5.2%. Literature mean values of $V_{dss}$ in premature infants range between 0.33 and 1.0 l kg$^{-1}$ [54], but no data seem to be available for term neonates. The early fall in the predicted curve reflects the rapid loss of excess ECW during the first months of life [12–14, 32, 34]. For midazolam literature data on $V_{dss}$ were more variable, but the model predictions fell mostly within the range of reported values. The high observed values at 4.7 and 5.2 years [58] are from children who were given midazolam during or after cardiac surgery, where plasma protein binding may have been compromised by bleeding and fluid replacement (as has been demonstrated for patients undergoing orthopaedic surgery [18]). The lower predicted $V_{dss}$ (in 1 kg$^{-1}$) in women than in men for theophylline, as well as the higher $V_{dss}$ for midazolam, is explained by the higher percentage of adipose tissue in the female body. Both findings are in agreement with literature data [18, 44, 65].

In addition, the calculated total CL of both drugs was in reasonable agreement with the literature, but as is evident from Figures 3 and 5, the intersubject variability in the experimental data was large. In the light of this and the problems of obtaining reliable data on $Q_H$ and hepatic enzyme activity (as discussed above), the present modelling cannot be regarded as definitive. Nevertheless, for theophylline the bias was quite low with a median underprediction of only 4.0%, and for midazolam the predictions generally fell within the mean ± SD of the literature values. Similarly as for $V_{dss}$, the CL values from the children who underwent cardiac surgery [58] are of questionable validity; impaired plasma protein binding should increase CL but drug interactions with premedication and anaesthesia may have lowered it.

The predicted renal excretion of theophylline (Table 4) also corresponded fairly well to experimental findings in infants and children; 18 ± 8.5% at 32 ± 15 weeks [50] and 12 ± 1.7% at 10-16 years of age [57]. In the neonatal period, the reported $f_e$ was higher than the calculated value, 55 ± 14% at 6.7 ± 2.7 weeks of life [50]. The discrepancy may be due to the fact that the modelling was based on changes in GFR and $f_u$ only. Urinary excretion in neonates may be higher than expected from changes in these parameters due to immaturity of tubular mechanisms, possibly resulting in a lower capacity to re-absorb theophylline [10].

Terminal $t_{1/2}$ is determined both by the CL and the $V_{dss}$ of the drug, and thus estimates of $t_{1/2}$ to some extent compound the errors in the estimation of the first two parameters. Thus for theophylline median bias and imprecision were higher for $t_{1/2}$ than for CL and $V_{dss}$, even though the largest prediction errors were due to the almost implausibly short half-lives reported for some of the 3–23-month-old infants [53]. Literature mean values of $t_{1/2}$ in premature infants range between 18 and 32 h,

whereas two term neonates showed half-lives of 14.5 and 8.5 h at 4 and 5 weeks of life [54]. For midazolam, the predictions for neonates and children were generally good, whereas $t_{1/2}$ in adults was over-predicted, due to the high calculated value for $V_{dss}$.

Predictions in the neonate are complicated by the rapid changes occurring after birth, not only in ECW, liver enzyme activity and kidney function, but also because of anatomical remodelling of the vasculature from the fetal to the autonomous circulation [12]. The neonatal period is thus one of change, not a certain time span during which pharmacokinetics can be predicted with some degree of certainty. As for the scaling in general, it is clear that the major changes with age affect clearance, both hepatic and renal, and these changes in the latter are the main reasons for changes in $t_{1/2}$. In comparison, changes in $V_{dss}$ with age were minor.

As in previous modelling studies [4, 5, 10, 24], the aim of the present work was to predict average or 'typical for age' values of pharmacokinetic parameters in the infant and child population. These can then be used for initial calculation of an appropriate dosing regimen. Deviations from the average CL and $t_{1/2}$ in individual patients, illustrated by the data scatters and error bars in Figures 3 and 5, cannot be predicted by any pharmacokinetic model since they are mainly due to interindividual variation in the liver CYP content.

In conclusion, a general PBPK model for the prediction of drug disposition over the age range neonate to young adult has been presented. Tables 1–3 constitute a reference source of physiological parameter values. Many of the values are only estimates or educated guesses. However, in many cases it seems unlikely that accurate data from humans of all ages will ever be available. The present compilations have as far as possible been validated internally. The utility of the model was demonstrated using two representative drugs with different physicochemical and pharmacokinetic characteristics, in terms of lipophilicity, $f_u$, tissue partitioning, hepatic extraction ratio and metabolism by CYP isoforms. In keeping with the aim to demonstrate the use of already available data, literature findings were utilized throughout. Future use of the PBPK model may further demonstrate its strengths and weaknesses.

*No special funding was sought or obtained for this study.*

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